Molecular Mapping and Confirmation of QTLs Associated with Oleic Acid Content in N00-3350 Soybean

Maria J. Monteros,* Joseph W. Burton, and H. Roger Boerma

ABSTRACT

The fatty acid composition of soybean [*Glycine max* (L.) Merr.] seed affects the flavor, nutritional value, and stability of the oil. Increasing oleic acid content in soybean oil would reduce the need for hydrogenation, a process that creates unhealthy *trans* fatty acids. The objective of this study was to map and confirm the areas of the soybean genome associated with oleic acid content from the soybean line N00-3350 (~583 g kg^{-1} oleic acid) using simple sequence repeat (SSR) markers. An $F_{2:3}$ population of 259 lines from the cross of G99-G725 × N00-3350 was used as a mapping population, and an $F_{2:3}$ population of 231 lines from the cross of G99-G3438 × N00-3350 was used for confirmation. Using single-factor analysis of variance, interval mapping, and composite interval mapping, six quantitative trait loci (QTLs) for oleic acid content were found on linkage groups LG-A1 (Satt211, R^2 = 4%), LG-D2 (Satt389, R^2 = 6%), LG-G (Satt394, R^2 = 13%), LG-G (Satt191, R^2 = 7%), LG-L (Satt418, R^2 = 9%), and LG-L (Satt561, R^2 = 25%) in the G99-G725 × N00–3350 population. All six QTLs for oleic acid were confirmed in the G99-3438 × N00-3350 population. The designations *cq*Ole-001, *cq*Ole-002, *cq*Ole-003, *cq*Ole-004, *cq*Ole-005, and *cq*Ole-006 have been assigned to these confirmed QTLs by the Soybean Genetics Committee. The identification of SSR markers linked to the oleic acid QTLs will facilitate the use of marker-assisted selection (MAS) in soybean breeding programs to increase the oleic acid content in soybean seed.

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Abbreviations: CIM, composite interval mapping; FDA, U.S. Food and Drug Administration; LG, linkage group; LOD, logarithm of odds; MAS, marker-assisted selection; MG, maturity group; MLG-MR, multiple linkage group regression analysis; PCR, polymerase chain reaction; QTL, quantitative trait locus; SF-ANOVA; single-factor analysis of variance; SLG-MR; single linkage group multiple regression analysis; SNP, single nucleotide polymorphism; SSR, simple sequence repeat.

The most common fatty acids found in the seeds of most plants, including soybean [*Glycine max* (L.) Merr.], belong to a small group of C16 and C18 fatty acids that include palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) (Somerville et al., 2000). The fatty acid composition of vegetable oils is variable and depends on the species (i.e., genetic factors) and environment during the growing season (Vles and Gottenbos, 1989). The content and relative proportions of each fatty acid are important factors because they affect the oil's flavor, stability, and nutritional value (Mensink et al., 1994). Soybean oil alone accounts for approximately 27% of the world's total edible oil production (Foreign Agricultural Service, 2002; Carter and Wilson, 1998). Global soybean oil consumption has increased at a steady rate of about 1 million Mg per year since 1994 (Wilson, 2004).

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Soybean oil used for human consumption is subject to U.S. Food and Drug Administration (FDA) guidelines for health claims made on labels. In accordance with the Nutritional Labeling and Education Act of 1990, FDA labeling regulations, require that a "low-saturated" vegetable oil have less than 7% total saturated fatty acids (U.S. Food and Drug Administration, 1999). Elite soybean cultivars produce, on average, 110 g kg^{-1} palmitic, 40 g kg^{-1} stearic, 230 g kg^{-1} oleic, 540 g kg^{-1} linoleic, and 80 g kg^{-1} linolenic acids (Wilcox et al., 1984; Diers et al., 1992; Schnebly and Fehr, 1993; Hui, 1996). Thus, soybean oil contains about 15% saturated fat, which is higher than both canola (*Brassica rapa* L.) and sunflower (*Helianthus annuus* L.) oils (Wilson et al., 2002). Soybean oil also contains a relatively high level of linoleic acid. The major limitation with vegetable oils that contain high concentrations of polyunsaturated fatty acids, like linoleic and linolenic acid, is flavor stability (Wilson, 1987). Both of these fatty acids may be oxidized, leading to undesirable odors and flavors (Crapiste et al., 1999; Mounts et al., 1988). Oleic acid, a monounsaturated fatty acid, is less susceptible to oxidation during storage and frying (Miller et al., 1987; Mercer et al., 1990).

The process of hydrogenation is currently used to improve the oxidative stability of soybean oil, which increases the shelf life of fats and foods prepared with it. However, this apparent solution to the stability issue may give rise to another problem. The bonds of unsaturated fatty acids in crude vegetable oils are predominately in a *cis* configuration. During hydrogenation, some double bounds may be rearranged into a *trans* configuration, where the hydrogen atoms end up on different sides of the chain (Wilson, 2004). These *trans* fatty acids can cause undesirable health effects, including elevated blood levels of low-density lipoproteins (LDL) and an increase in the risk for coronary heart disease (Willet, 1994; Hu et al., 1997; Lichtenstein et al., 1999; Mazur et al., 1999). These findings prompted the FDA to require that in addition to the saturated fat content, information on the amount of *trans* fatty acids must be included on the Nutrition Facts panel of a product's label (U.S. Food and Drug Administration, 2004), effective 1 Jan. 2006 (Center for Food Safety and Applied Nutrition, 2003). A viable alternative to increase the oxidative stability of the oil without hydrogenation would be to produce soybean oil with a more favorable fatty acid composition (i.e., 500 to 550 g kg^{-1} oleic acid and less than 30 g kg^{-1} linolenic acid) (Wilson et al., 2002).

Incorporation of a unique fatty acid composition into commercial cultivars would likely be enhanced by knowledge of the inheritance of the fatty acid composition in soybean. Oleic acid and polyunsaturated fatty acid content in soybean are quantitatively inherited (White et al., 1961; Burton et al., 1983; Carver et al., 1987). Yield trials of soybean lines with increased oleic acid content have shown that higher oleic acid content does not negatively affect yield (Carver et al., 1986).

Soybean oil use has been affected by the identification of mutations and naturally occurring variations affecting seed fatty acid synthesis (Palmer et al., 2004). In 1975, the use of mutation breeding with ethyl methane sulfonate or sodium azide was implemented, and changes in linolenic acid content were obtained (Hammond and Fehr, 1984). Genotypes with altered fatty acid profiles have been identified. For example, the line N97-3363-4 has recessive fatty acid desaturase alleles and contains about 60% oleic acid content. A number of lines possessing variant levels of fatty acids were used to develop the mid-oleic acid content line N00-3350 (Fig. 1). Selection for higher oleic acid content has been used to indirectly reduce linolenic acid. The level of linolenic acid in the line N78-2245 was reduced from 90 to 6 g kg^{-1} by selecting for oleic acid, which increased from 220 to 420 g kg^{-1}. Ancestors of N94-2473, a parent of N98-4445A and N00-3350, were selected for lower linolenic acid (Wilson et al., 2002). The lines N78-2245, N79-2077, N87-2122-4, and C1726 were selected for low palmitic acid content (Burton et al., 1994). The breeding line C1726 was developed by mutagenesis from the cultivar Century and has reduced palmitic acid levels (Wilcox et al., 1980; Erickson et al., 1988; Wilcox and Cavins, 1990). All of these lines selected for lower palmitic acid and lower linolenic acid content were used as parents in developing N98-4445A. The line N98-4445A belongs to maturity group (MG) IV and has 500 to 600 g kg^{-1} oleic acid content (Burton et al., 2006). N00-3350 is a single-plant selection from N98-4445A. N00-3350 and N98-4445A trace back to the same F_2 single plant and possess similar oleic acid content (Fig. 1).

Advances in DNA marker technology, including the development of simple sequence repeat (SSR) markers and the development of an integrated soybean genetic linkage map, have facilitated the genetic mapping of quantitative traits in soybean (Cregan et al., 1999). The most recent integrated genetic map for *G. max* contains over 1000 mapped SSR markers (Song et al., 2004). Twelve to 29 additional markers per linkage group were added to the earlier map of Cregan et al. (1999). The objectives of this study were to map and confirm the areas of the soybean genome that are associated with oleic acid content from N00-3350 (550 g kg^{-1} oleic acid) using SSR markers.

MATERIALS AND METHODS

Plant Material

An F_2 population consisting of 259 plants derived from the cross of G99-G725 (~206 g kg^{-1} 18:1) × N00-3350 (~620 g kg^{-1} 18:1) was developed for use as a mapping population. G99-G725 is a glyphosate-resistant backcross conversion of 'Boggs' (Boerma et al., 2000). Boggs is an MG VI cultivar with white flowers that

was derived from an F_5 plant from the cross G81-152 × 'Coker 6738'. G81-152 was derived from the cross D74-7741 × 'Coker 237'. D74-7741 is an MG VI breeding line selected from the cross of 'Forrest' × D70-3001. The F_2 population and seeds of each of the parents were planted in the Athens greenhouse on 16 Oct. 2001. Leaf tissue from each plant was collected for DNA extraction and at maturity F_2 plants were individually harvested.

A total of 231 F_2 plants from the cross G99-G3438 (~185 g kg^{-1} 18:1) × N00-3350 were used as a confirmation population. G99-G3438 is a glyphosate-resistant backcross conversion of 'Benning' (Boerma et al., 1997). Benning is a MG VII cultivar with purple flowers derived from an F_4 plant from the cross 'Hutcheson' × 'Coker6738'. The F_2 population and seeds of each parent were planted in the greenhouse on 17 June 2003. Leaf tissue from each plant was collected for DNA extraction and at maturity F_2 plants were individually harvested. In both populations, eight entries of each parent were randomized among the lines.

On 7 May 2004, the 259 $F_{2:3}$ lines from G99-G725 × N00-3350 and the 231 $F_{2:3}$ lines from G99-G3438 × N00-3350 were planted in 1-m row plots in Isabela, Puerto Rico. Three entries of each of the parents were planted in each experiment as checks. Plots were harvested individually on 2 Aug. 2004. In addition, 12 seeds each of 231 $F_{2:3}$ lines from the cross of G99-G3438 × N00-3350 were planted 25 May 2004 in 0.76-m × 0.45-m hills at the Univ. of Georgia Plant Sciences Farm, near Athens, GA. Hills were thinned to eight plants on 10 June 2004. Due to limited seed, a single replication was planted in the two locations. Individual plants from within some hills from G99-G3438 × N00-3350 grown in Athens matured at different times. For these hills, the early-maturing and late-maturing plants were harvested independently to create subsamples from each entry. Fatty acid analysis was determined separately for each early and late maturing subsample from each line.

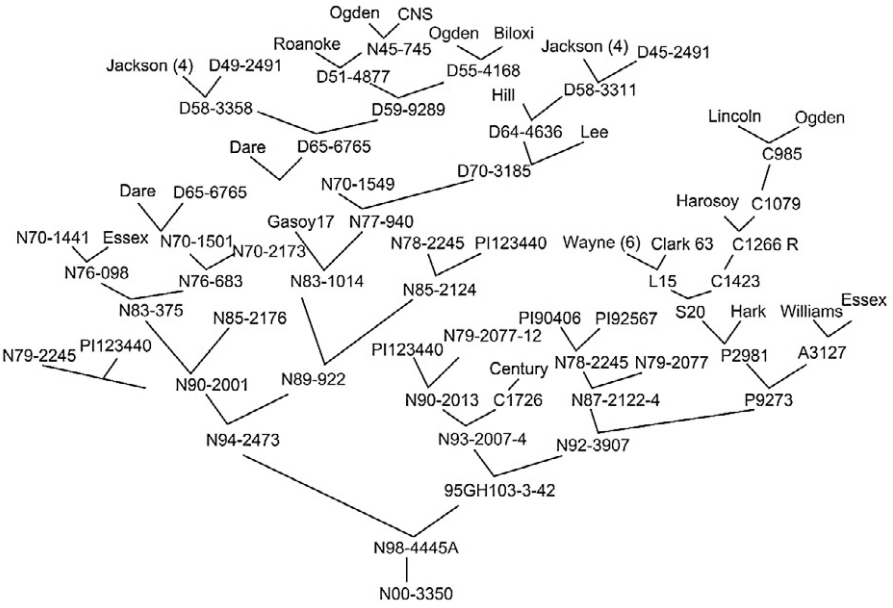

Figure 1. Pedigree of soybean line N00-3350.

Phenotypic Data

For the initial fatty acid determination of the G99-G725 × N00-3350 mapping population, $F_{2:3}$ seed produced in the Athens greenhouse was analyzed. If there were 35 $F_{2:3}$ seeds or more, a bulked random sample of 10 seeds from each plant was sent to USDA-ARS laboratories in Peoria, IL, and Raleigh, NC, for fatty acid analysis. If 18 to 34 seeds were available, 10 half-seed chips were sent to each laboratory. If fewer than 18 seeds were available, quarter-seed chips of each seed were sent to each laboratory. For the seeds produced in Puerto Rico, 10 seeds were sent to Peoria and 10 seeds to Raleigh for fatty acid determinations. Fatty acid content was determined using gas chromatography (Model 5890/6890, Hewlett-Packard, Palo Alto, CA) to evaluate methyl esters. Phenotypic data for each fatty acid were tested for a normal distribution using SAS (PROC UNIVARIATE PLOT) (SAS Institute, Cary, NC). The fatty acid phenotype of each entry used in the analysis is the average from the Peoria and Raleigh laboratories.

For the G99-G3438 × N00-3350 confirmation population, seeds were analyzed from the 2004 Athens, GA, and Isabela, PR, field locations. On the basis of seed availability, the following number of seeds were submitted for fatty acid analysis: (i) if >49 seeds were available, 15 seeds were sent to each laboratory for fatty acid analysis; (ii) if 40 to 50 seeds per line were available, 12 seeds per line were sent to each laboratory; (iii) if 29 to 39 seeds per line were available, 10 seeds per line were sent to each laboratory; and (iv) if 22 to 28 seeds were available, 8 seeds per line were sent to each laboratory. The fatty acid content of the 67 lines from G99-G3438 × N00-3350 that differed in maturity when planted in the field in Athens was determined separately for each subgroup. Most subgroups consisted of either four plants with early maturity and four plants with late maturity, three plants early and five plants late, or vice versa. In two cases, the early subgroup consisted of six plants and the late subgroup consisted of two plants.

SSR Analysis

Leaf tissue from each F_2 plant in the two populations was collected, lyophilized, and macerated. DNA was extracted using a modified CTAB (hexadecyltrimethylammonium acid) protocol (Keim et al., 1988), and resuspended in TE buffer. Polymerase chain reactions (PCRs) were similar to the protocol by Li et al. (2001), with some modifications. The 10-μL reaction mix contained 2 μL of 50 ng μL^{-1} template DNA, 1.0X PCR buffer, 2.5 mM $MgCl_2$, 100 μM of each dNTP, 0.2 μM each of the forward and reverse primers, and 0.5 unit of Promega *Taq* DNA polymerase (Madison, WI). Primers were labeled with the fluorescent dyes 6-FAM, NED, or HEX (PE-ABI, Foster City, CA). A 384-well or a 96-well GENE AMP PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA) was used for DNA amplification.

Pooled PCR products (3–4 μL) were combined with 2 μL deionized formamide, 0.75 μL loading buffer, and 0.2 μL Genescan ROX-500 internal size standard. The mixture was denatured at 95°C for 4 min., and 1 to 2 μL were loaded into

each of 96 lanes on 12-cm acrylamide:bisacrylamide (19:1) gels, using KLOEHN microsyringes (Kloehn Ltd., Las Vegas, NV). DNA amplicons were run on gels using an ABI Prism 377 DNA sequencer at 750 V for 1.5 to 2 h, with 1X TBE buffer. PE ABI 377 DNA sequencer collection software was used to collect the marker data (ABI, Foster City, CA). GeneScan Version 3.0 was used to analyze the DNA amplicons. Gels were scored with Genotyper Version 2.5 (ABI, Foster City, CA) and manually verified.

A genomewide screen of DNA markers was conducted using evenly spaced markers from all 20 linkage groups (Cregan et al., 1999). A total of 350 SSR markers covering all of the linkage groups were tested for polymorphism in the G99-G725 × N00-3350 population. A total of 180 SSR markers (51.4%) were polymorphic between the two parents. Markers evaluated for the confirmation population using 231 lines from the cross of G99-G3438 × N00-3350 were selected on the basis of significant associations with oleic acid content obtained from the mapping population. The early and late maturing subsamples from G99-G3438 × N00-3350 were genotyped separately. A random sample of six seeds from within each subgroup was planted in the Athens greenhouse on 10 Aug. 2005. Trifoliolate leaves for each subsample were collected for DNA extraction and SSR genotyping.

Data Analyses and Mapping

The correlation coefficient between the oleic acid data from the $F_{2:3}$ seed from G99-G725 × N00-3350 obtained from both laboratories and the single-factor analysis of variance for the marker analysis were calculated using SAS Version 8 (SAS Institute, 2001). For the oleic and linoleic acid content from Athens and Puerto Rico from the cross of G99-G725 × N00-3350, genotypes and environments were considered random effects and the genotype × environment interaction was used as an error term. The variance-component heritability based on the mean of one plot at two locations was calculated as $h^2 = \sigma^2_G/\sigma^2_{GE/2} + \sigma^2_G$, where σ^2_G = genotypic variance component and σ^2_{GE} = genotype × environment variance component (Fehr, 1987).

Linkage maps for all soybean linkage groups were constructed with MapManager QTX b20 (Manly et al., 2001) using the Kosambi mapping function (Kosambi, 1944). Single-factor analysis of variance (SF-ANOVA) was used to determine the significance of SSR genotypic class means using the general linear model (PROC GLM) SAS Version 8 (SAS Institute, 2001). An iterative process of single linkage group multiple regression analysis (SLG-MR) and multiple linkage group regression analysis (MLG-MR) using the STEPWISE selection criteria was used to identify the significant markers in the model associated with oleic acid content at the 5% significance level. Oleic acid content and marker data were analyzed to determine the presence and estimate the positions of QTLs using interval mapping with MapManager QTXb 20, and composite interval mapping (CIM) with QTL Cartographer V2.0 (Wang et al., 2005). For QTL detection, 1000 permutations were used to establish the minimum logarithm of odds (LOD) score. The CIM options and parameters used were similar to those described by Chung et al. (2003). A multiple regression model using SAS was also used for the two-factor analysis of variance to detect epistatic interactions between all pairs of significant markers. Additional information on the mapping methods used in this study has been described elsewhere (Lander and Botstein, 1989; Jansen, 1993; Jiang and Zeng, 1995).

RESULTS AND DISCUSSION

G99-G725 × N00-3350 Mapping Population

The correlation coefficient between the oleic acid percentages for the $F_{2:3}$ plants when grown in the greenhouse in Athens obtained from the laboratories in Peoria and Raleigh was 0.94 ($p < 0.001$). The fatty acid data are reported as an average for the data from the two laboratories (Table 1). The mean oleic acid content for the G99-G725 parent from Athens and Isabela was 206 g kg^{-1} and for the N00-3350 parent was 583 g kg^{-1}. The range of oleic acid content of the 259 $F_{2:3}$ lines in the mapping population was 228 to 613 g kg^{-1}, and the mean was 388 g kg^{-1}. There was no indication of transgressive segregation for low oleic acid among the progeny when compared to G99-G725. The $F_{2:3}$ line with the highest oleic acid content (613 g kg^{-1}) did exceed N00-3350. The heritability estimate for oleic acid content was 0.71 and for linoleic acid content was 0.54. The oleic heritability estimate is higher than a previous report from Hawkins et al. (1983) of 0.50 to 0.58, likely because a wider range in oleic acid content of the lines was evaluated in the present study.

The number of markers per linkage group (LG) ranged from 4 on LG-J to 15 on LG-G (data not shown). Sixteen SSR markers on LG-J were screened for polymorphism between the mapping parents, but only four of them were polymorphic. A minimum of six markers per linkage group was used for the construction of linkage maps for the remaining soybean linkage groups. Via SF-ANOVA, significant markers associated with oleic acid content were found on LGs A1, D2, G, and L (Table 2). Linkage maps with additional markers on LG-A1, D2, G, and L were constructed (Fig. 2). The order of the markers in these LGs based on our data is in close agreement with that of the integrated soybean genetic linkage map (Cregan et al., 1999; Song et al., 2004).

On the basis of SF-ANOVA, five markers on LG-A1 near Satt200 were significantly ($p < 0.01$) associated with oleic acid content. These markers were located in the 85 to 96 cM region on the USDA–ARS soybean consensus map. Using SLG-MR, only marker Satt211 was retained in the model (Table 2). Interval mapping shows the QTL likelihood plot near Satt200 and Satt599 (Fig. 2). Using the markers found significant by SF-ANOVA on LG-D2, the SLG-MR retained Satt389, which explained 4% of the variation in the oleic acid content. The LOD peak for oleic acid content based on interval mapping was located near Satt389 (Fig. 2b).

Table 1. Mean fatty acid content of the parents and mean range of progeny from the G99-G725 × N00–3350 soybean population grown in an Athens, GA, greenhouse and in the field at Isabela, PR. The values reported are an average from the two locations.

Line	Palmitic	Stearic	Oleic	Linoleic	Linolenic
			g kg^{-1}		
G99-G725	113	27	206	568	87
N00-3350	86	35	583	280	36
$F_{2:3}$ mean	101	28	388	432	51
$F_{2:3}$ range	81.5–124.5	19.5–39	228–613	230–579	27–109
$LSD_{0.05}$†	3.3	2.8	29.7	24.2	4.8

†For comparison of individual $F_{2:3}$ lines.

On LG-G, eight SSR markers were found to be associated with the oleic acid content on the basis of SF-ANOVA ($p < 0.001$) (Table 2). When these markers were placed in an SLG-MR equation, the markers Satt394 (43.4 cM), Satt594 (52.9 cM), and Satt303 (53.4 cM) had the largest overall effect with an R^2 value of 13%. When LG-G was evaluated by interval mapping, three peaks were identified (Fig. 2c). One peak was near Satt235, one at Satt394, and one near Satt191.

On the basis of SF-ANOVA, seven markers on LG-L were associated with the variation in oleic acid content ($p < 0.001$). Of these seven markers, only Satt418 and Satt156 were retained in the LG-L SLG-MR (Table 2). Using interval mapping, three QTLs associated with oleic acid were identified (Fig. 2d). One QTL was located near Satt418, the second QTL on LG-L was approximately 40.5 cM away near Satt561, and a third QTL was identified between Satt418 and Satt561. Using SF-ANOVA, Satt418 explains 9% of the variation, Satt561 explains 25% of the variation, and Satt156 explains 15% of the variation (Table 2). Schlueter et al. (2007) identified a tandem duplication of FAD2-2 genes on LG-L, suggesting that a FAD2 gene could be responsible for the effect of the oleic acid QTLs identified in our study. Modifier QTLs for oleic acid have also been reported on LG-L in the 68.5 to 89.1 cM region (Hyten et al., 2004a).

Composite interval mapping was used to determine whether the significant effects at several linked markers or intervals were independently conditioning oleic acid content. A limited number of background markers were identified via the forward–backward stepwise regression option of QTL Cartographer V 1.3 using conservative probability thresholds (P*in* = 0.01; P*out* = 0.01). A 1-cM window parameter was used to exclude from the background marker group any marker located within 1 cM of the two markers flanking any interval being tested for a putative QTL peak (Chung et al., 2003). The CIM of oleic acid QTL on LG-L identified Satt561 as a background marker and the LOD peak at Satt418 surpassed the significance threshold determined by 1000 permutation tests, indicating the presence of two independent QTLs for oleic acid content on this LG

Table 2. Simple sequence repeat markers associated with the mean oleic acid content in 259 soybean lines from G99-G725 × N00-3350 grown in the Athens, GA, greenhouse and the Isabela, PR, field.

LG	cM	Marker	SF-ANOVA†		Single LG MR‡	Multiple LG MR
			R^2	2a	Partial R^2	Partial R^2
			%	g kg^{-1}	%	
A1	85.6	Satt599	6**	53.3		
A1	92.9	Satt200	5**	44.2		
A1	93.2	Satt236	4**	39.6		
A1	95.2	Satt225	4**	43.2		
A1	96.0	Satt211	4**	42.3	4**	8***
D2	79.2	Satt389	6***	56.2	4***	4**
D2	84.6	Satt311	7***	56.1		
D2	85.1	Satt226	8***	42.5		
G	21.9	Satt235	8***	61.8	8***	8***
G	43.4	Satt394	13***	76.8		
G	47.3	Satt501	10***	57.8		
G	52.9	Satt594	13***	54.0		
G	53.4	Satt303	13***	62.5		
G	76.8	Satt288	8***	44.5		
G	80.4	Satt612	7***	41.9		
G	96.6	Satt191	7***	47.1		4***
L	30.2	Satt143	7**	46.3		
L	30.9	Satt418	9***	55.4	3**	3**
L	34.5	Satt313	8***	52.7		
L	56.1	Satt156	15***	72.6	7***	
L	66.5	Satt166	8***	71.0		
L	70.4	Satt527	10***	62.3		
L	71.4	Satt561	25***	78.8		3*
					TOTAL	30.0

** $p < 0.01$.

*** $p < 0.001$.

†SF-ANOVA, single-factor analysis of variance; R^2, % of the total trait variance explained by the genotype at a marker locus; 2a, the difference in oleic acid content at a simple sequence repeat marker homozygous for the N00-3350 allele vs. homozygous for the G99-G725 allele.

‡MR, multiple regression analysis including significant markers within each linkage group and across linkage groups.

(Fig. 3b). The CIM on LG-G revealed a similar pattern to that on LG-L. The oleic acid QTL near both Satt394 and Satt191 exceeded the LOD threshold. However, using CIM the QTL near Satt235 did not exceed the significance threshold (Fig. 3a). Similar to the results obtained using interval mapping, the QTL likelihood plots of the oleic acid QTL on LG-A1 and on LG-D2 using CIM locate the position of the QTL near Satt599 and near Satt311, respectively (data not shown).

A STEPWISE multiple regression analysis across the four LGs with significant SSR markers associated with oleic acid using 259 lines from G99-G725 × N00-3350 indicated that QTLs linked to Satt211 (R^2 = 8%) on LG-A1, Satt389 (R^2 = 4%) on LG-D2, Satt235 (R^2 = 8%) and Satt191 (R^2 = 4%) on LG-G, and Satt418 (R^2 = 3%), and Satt561 (R^2 = 3%) on LG-L contribute to the oleic

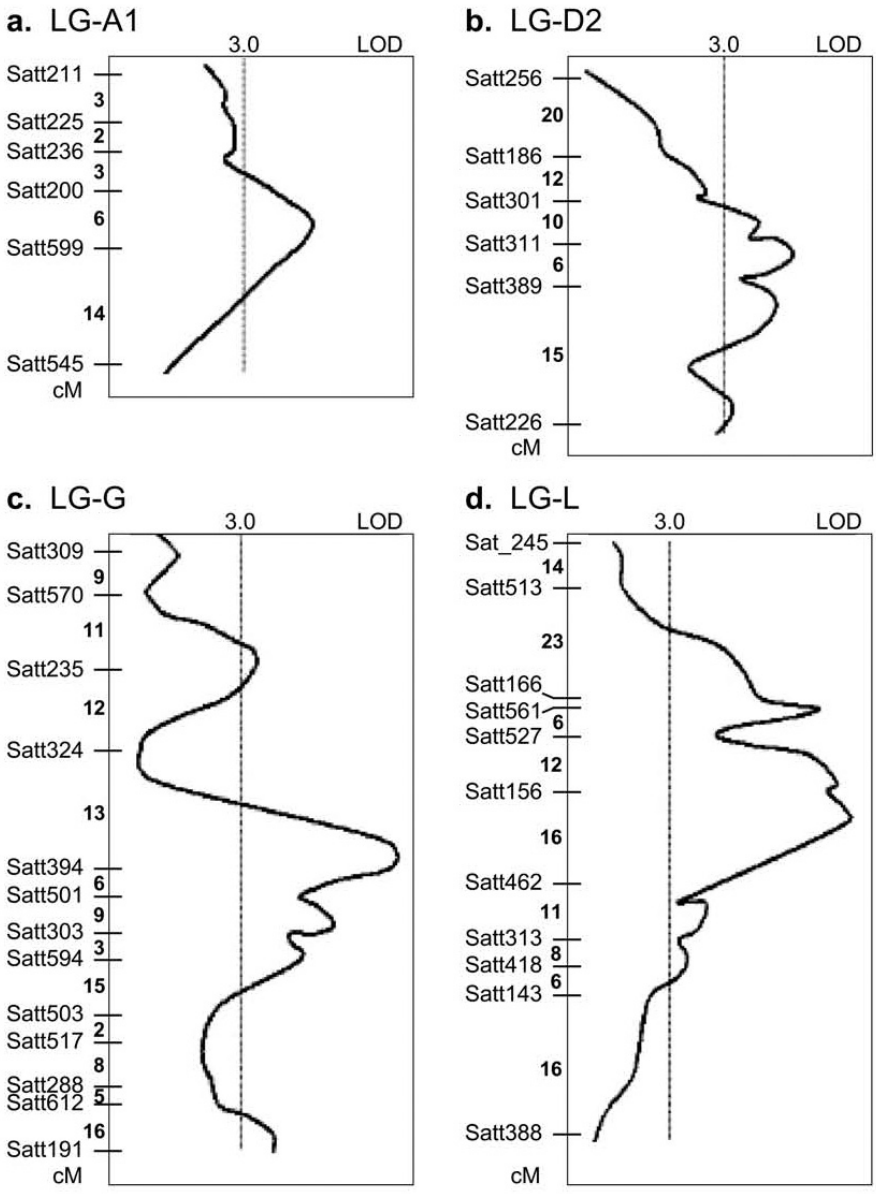

Figure 2. Quantitative trait locus (QTL) likelihood plots from interval mapping for mean oleic acid QTL using 259 soybean lines from the G99-G725 × N00-3350 population grown in the Athens, GA, greenhouse and Isabela, PR, field. For each linkage group (LG), the permutation-derived (n = 1000 per trait) logarithm of odds (LOD) score significance criteria are indicated by a vertical dotted line at 3.0.

acid content and together explain 30% of the variation in oleic acid content (Table 2). To evaluate possible epistatic interactions, all pairs of significant markers were tested for interaction using a two-factor ANOVA (SAS Institute, Cary, NC). None of the two-marker interactions were significant ($p \leq 0.01$).

When all six putative independent QTLs associated with oleic acid content identified using SF-ANOVA, interval mapping, and CIM were considered individually, the N00-3350–derived allele increased oleic acid content at each QTL (Table 2). When the six markers were homozygous for the N00-3350 allele, the predicted mean for oleic acid content increased by 356 g kg^{-1} compared with those having the G99-G725 allele. In most cases, except for the QTL near Satt418, the relative oleic acid content between the homozygous and the heterozygous genotypes indicated additive gene action for the alleles at these QTLs (Table 3). For the QTL near Satt418, the N00-3350 allele for increased oleic acid was recessive. Although this work explained a considerable amount of genetic variation, additional variation in oleic acid content due to effects unaccounted for by the markers, epistasis, and the genotype × environment interaction remains. Transgressive segregation in the progeny for oleic acid content suggests the possibility that positive alleles for oleic acid content are also present in G99-G725.

In sunflower, Schuppert (2004) showed that the Ol_1 mutation associated with FAD2-1 is necessary but not sufficient to produce the high oleic acid phenotype, presumably because additional QTLs segregate in some of the genetic backgrounds evaluated. Additionally, when a segregating population was evaluated, the data indicated that the oleic acid phenotype was caused by the main effect and the interaction of several genes. When a biosynthetic pathway is involved, it is likely that the availability of a substrate and the activity of genes upstream in the pathway may affect the accumulation of individual components downstream in the pathway. These findings provide support for the complexity of the oleic acid phenotype in the N00-3350 soybean line.

Several of the oleic content QTLs identified in this study are located in the same genomic regions as previously reported oil QTLs. Oil content QTLs were reported on LG-A1 in the interval delimited by Satt174 (88.6 cM) to B170_1 (94.9 cM) using two different populations (Brummer et al., 1997; Orf et al., 1999; Mansur et al., 1996; Specht et al., 2001). On LG-D2, an oil content QTL was reported near Satt082 (87.2 cM) (Hyten et al., 2004a). Quantitative trait loci for oil content have also been reported on LG-G (Brummer et al., 1997; Lee et al., 1996). Five different studies reported oil content QTLs on LG-L, one of which is closely associated with Satt166 (66.5 cM, R^2 = 8%) (Diers et al., 1992; Lee et al., 1996; Hyten et al., 2004b, Mansur et al., 1996; Orf et al., 1999).

The mid-oleic QTLs identified in this study were also associated with the variation in linoleic acid content (Table 4). Oleic acid and linoleic acid were found to be negatively

correlated, with a correlation coefficient of $r = -0.91$ ($p < 0.001$). Previous studies similarly showed oleic acid and linoleic acid levels to be negatively correlated (Howell et al., 1972; Burton et al., 1983). The additive effects for the N00-3350 alleles for linoleic acid content were negative, indicating that the N00-3350 allele contributed to a decrease in linoleic acid content and, as previously shown, an increase in oleic acid content. Biochemical evidence indicates that linoleic acid and linolenic acid are produced by the consecutive desaturation of oleic acid (Howell et al., 1972; Wilson et al., 1981). Therefore, a reduction in linoleic acid content is likely when its precursor, oleic acid, is increased in the seed.

None of the markers associated with oleic acid on LG-A1, D2, G, and L explained any of the variation in the linolenic acid content. However, Satt318 on LG-B2 explained 12% of the variation in linolenic acid content, $p < 0.001$ (data not shown). At this QTL, N00-3350 is contributing an allele that decreases the amount of linolenic acid in soybean seeds. These results are consistent with a previous study in which the *fan* locus controlling reduced linolenic acid was mapped to this region of LG-B2 (Brummer et al., 1995).

Simple sequence repeat markers on LG-A1, D2, G, and L are also significant for palmitic acid content (Table 4). A QTL near Satt211 on LG-A1 explains 4% of the variation in palmitic acid content. A study conducted by Li et al. (2002) indicated that N87-2122-4 contributed an allele for a major QTL for palmitic acid on LG-A1, which explained on average 34% of the variation in palmitic acid content. This QTL was located near the top of LG-A1, which is more than 90 cM from the oleic acid QTL. An association between palmitic acid and Satt166 on LG-L was also found in a cross of Cook × C1726 (David Hulburt, personal communication, 2006). In the same study, Satt458 on LG-D2 was also found to be associated with palmitic acid content.

G99-G3438 × N00-3350 Confirmation Population

The mean oleic acid content in the Puerto Rico environment was 186 g kg^{-1} for the G99-G3438 parent and 668 g kg^{-1} for the N00-3350 parent (Table 5). The range for oleic acid content in the 231 $F_{2:3}$ lines from G99-G3438 × N00-3350 was 212 to 635 g kg^{-1}, and their progeny mean was 409 g kg^{-1}. In the Puerto Rican environment, none of the $F_{2:3}$ lines produced higher levels of oleic acid than N00-3350 or less oleic acid than G99-G3438. Data from the SF-ANOVA analysis of the 231 $F_{2:3}$ lines from G99-G3438 × N00-3350 indicated that five of the QTLs associated with oleic acid content in the G99-G725 × N00-3350 mapping population were also significant ($p = 0.05$) in this population (Table 6). The putative oleic QTL located on LG-A1 (Satt211),

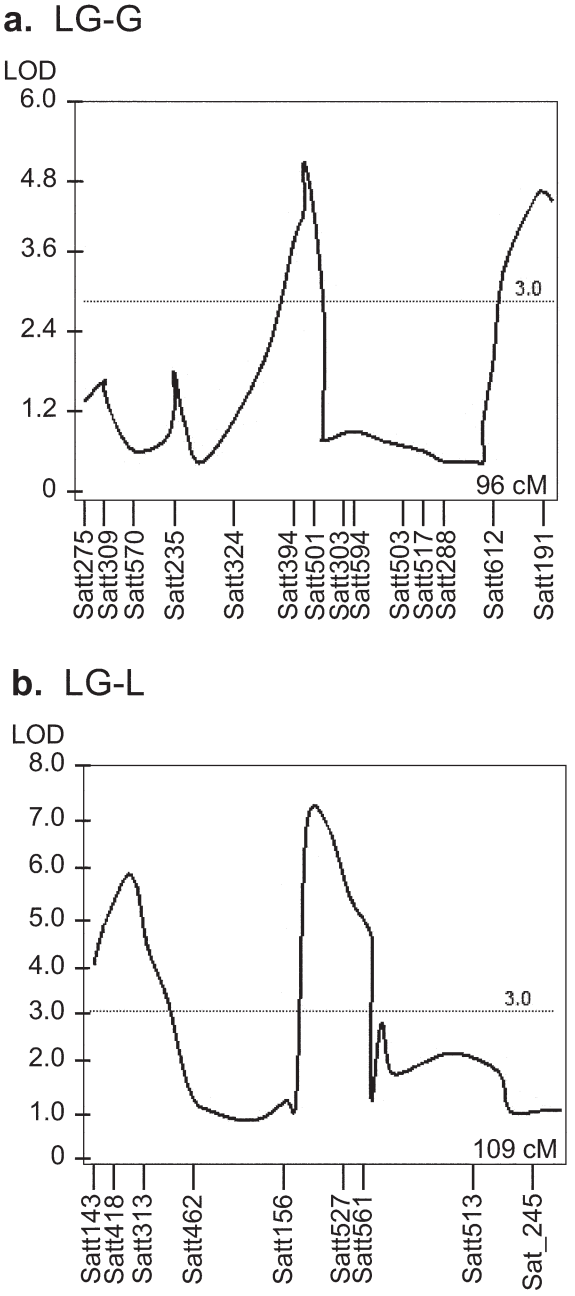

Figure 3. Composite interval mapping for mean oleic acid quantitative trait loci using 259 soybean lines from G99-G725 × N00-3350 grown in the Athens, GA, greenhouse and Isabela, PR, field. The dashed line shows the logarithm of odds (LOD) plot when background markers were used. (a) LG-G. (b) LG-L. The permutation-derived (n = 1000 per trait) LOD significance criteria are indicated by a dashed horizontal line at 3.0.

two on LG-G (Satt394 and Satt191), and one on LG-L (Satt561) were significant when oleic acid content was measured in the Puerto Rican environment. However, the amount of variation in oleic acid content explained by these QTLs was lower than in the mapping population (Table 2). The QTL on LG-D2 near Satt226 was not significant in this environment.

In the G99-G3438 × N00-3350 population, segregation for maturity date across and within lines was

Table 3. Mean oleic acid content for simple sequence repeat markers associated with putative oleic acid quantitative trait loci (QTLs) in 259 soybean lines from G99-G725 × N00-3350 grown in the Athens, GA, greenhouse and Isabela, PR, field.

Oleic QTL	LG†	Marker	GG‡	GN‡	NN‡
			g kg^{-1}		
1	A1	Satt211	365.6 (58)§	384.6 (122)	409.0 (71)
2	D2	Satt389	357.9 (49)	383.4 (121)	412.0 (64)
3	G	Satt394	353.6 (56)	383.9 (132)	430.5 (54)
4	G	Satt191	351.4 (62)	382.9 (118)	401.1 (55)
5	L	Satt418	371.2 (50)	377.7 (148)	427.6 (57)
6	L	Satt561	356.4 (67)	386.3 (134)	436.0 (52)

†LG, linkage group.

‡GG = homozygous for the allele from G99-G725; NN = homozygous for the allele from N00-3350; GN = heterozygous for the alleles from G99-G725 and N00-3350

§Number of $F_{2:3}$ lines in each class is shown in parentheses.

observed in the field in Athens, GA. For the 67 lines containing plants with more than a 7-d range in maturity, the plants were separated into early and late subsamples. Fatty acid determination for these subsamples was made separately for the 67 lines. The analysis of maturity date subsamples included only the data from Athens, since only minor differences in maturity (less than 4 d) among the lines were observed in Puerto Rico. The subsamples from these lines were genotyped separately with SSR markers on LGs where putative oleic acid QTLs had been identified. In this case, the oleic acid QTL near Satt561 was significant across all the lines regardless of whether the data from the early or the late maturing subsamples were considered in the analysis (Table 7). The marker Satt397, which is 9.9 cM from Satt389 on the consensus map, suggests that this putative QTL on LG-D2 is significant. Additionally, a single nucleotide polymorphism (SNP) marker obtained from a soybean Fad2 sequence mapped to the same location as the oleic acid QTL on LG-D2 (Monteros, unpublished data, 2007). Consistent with the mapping population, at all of these QTLs, the N00-3350 alleles were associated with increased oleic acid content.

In the SF-ANOVA analysis, Satt418 on LG-L was not significant either with or without the lines that had different maturity dates (Table 6, Table 7). Satt153 on LG-O was significant in the subsamples from G99-G3438 × N00-3350. However, Satt153 also explained 9% of the variation observed in maturity dates. A recent study mapped the location of the FAD2-1A gene to this region in LG-O (Schlueter et al., 2007), suggesting that a fatty acid desaturase gene could be responsible for the oleic acid QTL identified in this linkage group. The parents used in the mapping and confirmation population belong to different maturity groups. Benning, the recurrent parent of G99-G3438 is an MG VII cultivar, and Boggs, the recurrent parent of G99-G725, is an MG VI cultivar. In an SF-ANOVA analysis with SSR markers on LGs previously associated with oleic acid content, Satt561 was significantly associated with maturity date ($p < 0.001$). However, Satt418 was not significantly associated with maturity date (data not shown). Satt501, which is located 16 cM from Satt418 on the consensus map, is significantly associated with oleic acid content in this population. Evaluation of oleic acid content in the greenhouse and in Puerto Rico allowed us to compress the range in maturity of the populations and identify QTLs affecting oleic acid content. The difficulties of mapping oleic QTLs in populations with wide ranges in maturity was recently shown in the research of Bachlava et al. (2008).

The results from genotyping G99-G725 and G99-G3438 with SSR markers on LG-D2 and LG-L reveal different allele sizes (data not shown). These results suggest that differences in genetic background, in addition to the differences in maturity date, may be affecting the oleic acid QTLs. Studies in *Brassica* reported significant effects of genetic background on the content of major target fatty acids (Tang and Scarth, 2004). Studies with soybean near-isogenic lines in which background differences are minimized could be useful in evaluating the individual and combined effects of the oleic acid QTLs identified.

The size of the mapping population allowed sufficient statistical power to be able to detect QTLs with relatively small effects, which would have gone undetected using a smaller sample size or whose effects would have likely been overestimated. The oleic acid QTLs near Satt211 on LG-A1, Satt389 on LG-D2, Satt394 and Satt191 on LG-G, and Satt418 and Satt561

Table 4. Single-factor ANOVA for markers associated with soybean fatty acid content in G99-G725 × N00-3350 grown in the Athens, GA, greenhouse and Isabela, PR, field.

LG†	SSR†	cM	Palmitic		Stearic		Linoleic		Linolenic	
			R^2‡	2a‡	R^2	2a	R^2	2a	R^2	2a
			%	g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}
A1	Satt599	85.6	1*	2	ns§		5***	–41	ns	
A1	Satt225	95.2	2*	3	ns		6***	–44	ns	
A1	Satt211	96.0	4***	4	ns		5***	–42	ns	
D2	Satt311	84.6	2*	–3	ns		6***	–46	ns	
D2	Satt226	85.1	ns		ns		5***	–41	ns	
G	Satt235	21.9	3**	–4	ns		4***	–43	ns	
G	Satt394	43.4	6***	–5	ns		9***	–57	ns	
G	Satt191	96.6	4***	–4	ns		6***	–43	ns	
L	Satt418	30.9	2*	–3	ns		5***	–45	ns	
L	Satt561	71.4	4***	–4	ns		8***	–55	ns	

*$p < 0.05$.

**$p < 0.01$.

***$p < 0.001$.

†LG, linkage group; SSR, simple sequence repeat.

‡R^2, % of the total trait variance explained by the genotype at a marker locus; 2a, the difference in oleic acid content at an SSR marker homozygous for the N00-3350 allele versus homozygous for the G99-G725 allele.

§NS, nonsignificant marker association.

Table 5. Mean fatty acid content of parents and the mean range of 231 soybean progeny lines from the G99-G3438 × N00-3350 population used for confirmation. The values reported for the parental lines are an average of 16 samples per entry (two locations with eight samples per location).

Line	Palmitic	Stearic	Oleic	Linoleic	Linolenic
	g kg^{-1}				
Athens, GA, 2004					
G99-G3438	120	38	185	565	92
N00-3350	83	32	631	231	23
$F_{2:3}$ mean	103	34	395	424	44
$F_{2:3}$ range	79–128	25–54	226–649	207–574	27–74
$LSD_{0.05}$†	2.8	2.3	23.6	19.5	2.6
Isabela, PR, 2004					
G99-G3438	124	32	186	588	69
N00-3350	85	28	668	200	20
$F_{2:3}$ mean	108	26	409	419	38
$F_{2:3}$ range	81–131	20–37	212–635	216–590	21–67
$LSD_{(0.05)}$ †	2.7	1.7	8.8	7.3	1.7

†For comparison of individual $F_{2:3}$ lines.

Table 6. Marker regression analysis for oleic acid content using 231 soybean lines from G99-G3438 × N00-3350 grown in Isabela, PR.

QTL†	LG†	cM	Marker	Puerto Rico 2004	
				R^2‡	2a‡
				%	g kg^{-1}
1	A1	85.6	Satt599	2*	37
	A1	96.0	Satt211	3**	45
2	D2	85.1	Satt226	ns§	
3	G	43.3	Satt394	3*	43
4	G	96.6	Satt191	2*	41
5	L	30.9	Satt418	ns	
	L	47.3	Satt501	7***	70
6	L	71.4	Satt561	2**	36
	L	106.4	Satt513	ns	

*$p < 0.05$.

**$p < 0.01$.

***$p < 0.001$.

†QTL, quantitative trait locus; LG, linkage group.

‡R^2, % of the total trait variance explained by the genotype at a marker locus; 2a, the difference in oleic acid content at a SSR marker homozygous for the N00–3350 allele versus homozygous for the G99-G725 allele.

§NS, nonsignificant marker association.

on LG-L were confirmed across different environments and in two independent populations. Overall, six putative QTLs for oleic acid content were identified and confirmed in an independent population. These results indicate that considerable progress can be made through selection of the N00-3350 alleles at the identified oleic acid QTLs to obtain soybean oil with higher oleic acid content. Although the N00-3350 alleles at these oleic QTLs will produce higher oleic acid content than lines with G99-G725 or G99-G3438 alleles at these QTLs, the absolute oleic acid level of a line will be influenced by the environment (Oliva et al., 2006).

The designations Ole1-1 through Ole1-6 have been assigned in Soybase (http://soybase.org/) to oleic acid QTLs identified from a cross between A81356022 × PI468916 (Diers and Shoemaker, 1992). The designation *cq* for "confirmed QTL" has been proposed as a way to allow breeders and researchers to recognize these QTLs as having been mapped and confirmed in a population derived by independent meiotic events (Fasoula et al., 2004). Knowledge of which QTLs have been confirmed can allow breeders and researchers to prioritize the QTLs they want to incorporate in their programs. Therefore, we propose to designate the confirmed oleic acid QTLs on LG-A1 near Satt211 as *cq*Ole-001, on LG-D2 near Satt389 as *cq*Ole-002, on LG-G near Satt394 as *cq*Ole-003, on LG-G associated with Satt191 as *cq*Ole-004, on LG-L near Satt418 as *cq*Ole-005, and on LG-L near Satt561 as *cq*Ole-006. These QTL designations have been approved by the

Table 7. Marker associations with oleic acid content from the subsamples from soybean G99-G3438 × N00-3350 in Athens, GA. The early (E) and late (L) subsamples were separately genotyped and analyzed for fatty acid content.

LG†	cM	Marker	163 lines without E and L		163 lines + 67 early lines		163 lines + 67 late lines	
			R^2‡	2a‡	R^2	2a	R^2	2a
			%	g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}
A1	85.6	Satt599	ns§		ns		ns	
	96.0	Satt211	ns		ns		ns	
D2	69.3	Satt397	2*	39	ns		ns	
	85.1	Satt226	ns		ns		ns	
	84.6	Satt311	5**	46	4*	43	3*	42
	93.7	Satt301	ns		ns		ns	
G	21.9	Satt235	ns		ns		ns	
	43.4	Satt394	ns		ns		ns	
	96.6	Satt191	ns		ns		ns	
L	30.9	Satt418	ns		ns		ns	
	66.5	Satt166	4**	43	5***	45	6***	52
	70.4	Satt527	ns		ns		ns	
	71.4	Satt561	4*	44	7***	54	7***	58
	106.4	Satt513	3*	47	4**	44	12***	74
	115.1	Sat_245	ns		2*	29	2*	28
O	118.1	Satt153	7**	88	5**	49	9***	63

*$p < 0.05$.

**$p < 0.01$.

***$p < 0.001$.

†LG, linkage group.

‡R^2, % of the total trait variance explained by the genotype at a marker locus; 2a, the difference in oleic acid content at a SSR marker homozygous for the N00-3350 allele versus homozygous for the G99-G725 allele.

§NS, nonsignificant marker association.

Soybean Genetics Committee (http://www.soygenetics.org/committee.php).

The quantitative nature of the oleic acid trait makes phenotypic selection of the mid-oleic acid content a challenging task. Molecular markers can be used to identify the number, location, and contribution of QTLs that affect oleic acid content in soybean oil. One type of breeding strategy to incorporate all oleic acid QTLs would involve conducting two separate backcross populations to introgress the oleic acid QTLs from N00-3350. In each backcross population, three of the oleic QTLs would be introgressed. At the final step in the program, the two backcross populations would be crossed and marker-assisted selection (MAS) used to select a line with all six oleic QTLs. Increasing the number of transferred segments also increases the risk of inadvertently introgressing undesirable agronomic trait alleles at loci on linked donor chromosomal segments (Stuber et al., 1999). Therefore, the two QTLs on LG-G and LG-L should be selected for in different backcross populations to maximize the genetic contribution of the elite parent. This strategy would reduce the potential for linkage drag by introgressing a smaller genomic region from N00-3350. Although no QTLs conditioning traits of agronomic importance, such as seed shattering or lodging, are reported on LGs where oleic acid QTLs have been found, some germination problems and potential shattering have been associated with the N00-3350 line (data not shown).

The presence of six QTLs for oleic acid content may reduce the effectiveness of MAS when compared to a trait determined by relatively few QTLs with major effects. However, as new fatty acid gene-based SNP markers become available, it may be possible to increase the mapping resolution of the oleic acid QTLs identified in this study and allow an increased throughput in future MAS applications.

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